EPA Reviewer: Linda Taylor, Ph.D. **Signature:** _____
Risk Assessment Branch VII, Health Effects Division (7509P) Date_____
EPA Secondary Reviewer: Elissa Reaves, Ph.D. **Signature:** _____
Risk Assessment Branch VI, Health Effects Division (7509P) Date_____

TXR#: 0055372

DATA EVALUATION RECORD

STUDY TYPE: Non-guideline; Effects on Brain and RBC Cholinesterase in Adult and Juvenile Rats

PC CODE: 098301 **DP BARCODE:** D376136

TEST MATERIAL (PURITY): Aldicarb (99.8% a.i.)

SYNONYMS: Temik; 2-methyl-2-(methylthio) propionaldehyde-0-(methylcarbamoyl) oxime

CITATION: Beck, M. J. (2010). A Dose-Response Study of Red Blood Cell and Brain Cholinesterase in Adult and Juvenile Rats Following Oral (Gavage) Administration of Aldicarb. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Project ID: WIL-21209, February 19, 2010. MRID 47994305. Unpublished.

Beck, M. J. (2010). A Time Course Study of the Effects of Aldicarb on Red Blood Cell and Brain Cholinesterase in Adult and Juvenile Rats Following Oral (Gavage) Administration. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Project ID: WIL-21208, February 19, 2010. MRID 47994304. Unpublished.

Beck, M. J. (2009). A Comparative Dose Study of the Effects of Aldicarb on Red Blood Cell and Brain Cholinesterase in Adult and Juvenile Rats Following Oral (Gavage) Administration. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Project ID: WIL-21210, November 19, 2009. MRID 47994303. Unpublished.

Beck, M. J. (2009). A Dose-Range Finding Study of the Effects of Aldicarb on Red Blood Cell and Brain Cholinesterase in Adult and Juvenile Rats Following Oral (Gavage) Administration. WIL Research Laboratories, LLC, Ashland, OH. Laboratory Project ID: WIL-21207, November 19, 2009. MRID 47994302. Unpublished.

SPONSOR: Bayer CropScience LP, Research Triangle Park, NC

EXECUTIVE SUMMARY - This series of non-guideline cholinesterase inhibition studies (MRID 47994302-47994305) was undertaken to evaluate any differences between postnatal day 11 (PND 11) pups and adult rats with regard to cholinesterase inhibition.

Dose Range-Finding Study (MRID 47994302): This study was performed to determine the dose levels for use in the time to peak effect study (MRID 47994304). Adult Crl: CD (SD) rats (0.1 or 0.3 mg/kg) and PND 11 pups (0.025, 0.05, or 0.1 mg/kg) were administered single doses of aldicarb in deionized water *via* gavage (6/sex/dose for both age groups). At 60 minutes post dose, cholinesterase activity was assessed in the RBC and brain compartments.

Results: Slight whole body tremors were observed in the adult rats at 0.3 mg/kg and in PND 11 pups at 0.1 mg/kg. A dose-related reduction in cholinesterase activity (both compartments) was observed at all dose levels in both sexes and age groups. The dose levels selected from this study for the time-to-peak-effect study were 0.08 mg/kg (adult rat) and 0.01 mg/kg (PND 11 pup). Following consultation with HED, a second dose level for the PND 11 pups of 0.02 mg/kg was included in the time-course study.

Comparative Dose-Study (MRID 47994303): This comparative study was to compare the amount of RBC or brain ChE inhibition in adult or PND11 male Crl: CD (SD) rats after a single gavage dose. Aldicarb in deionized water was administered once *via* gavage (5 ml/kg) to 6 adult male and 6 PND11 male Crl: CD (SD) rats/sex/dose of 0, 0.01 or 0.04 mg/kg. Adult rats were euthanized approximately 40 minutes and juvenile rats at 60 minutes post-dosing, times that were considered to be the peak-effect times for each age group.

Results: There were no clinical findings at 20 minutes and 40 minutes (adult males) or 20 and 60 minutes (juvenile males) following dose administration. There were no changes in RBC and whole brain cholinesterase activity in adult males at either dose level. In PND 11 males, RBC cholinesterase activity in the 0.01 mg/kg and 0.04 mg/kg groups was 32% and 74% lower, respectively, compared to PND 11 control males at approximately 60 minutes post-dosing. Whole brain cholinesterase activity in PND 11 males was 36.7% lower at 0.04 mg/kg than control PND 11 males. There were no significant differences on mean brain weights in adult or PND 11 males at either dose level.

Time-Course Study (MRID 47994304): Aldicarb (99.8% a.i.; Lot #: 1218200307) in deionized water was administered once *via* gavage (5 mL/kg) to 6 adult and 6 PND 11 Crl: CD (SD) rats/sex/dose/sacrifice time at doses of 0 or 0.08 mg/kg (adult rats), and 0, 0.01 mg/kg, or 0.02 mg/kg (PND 11 pups) to determine the time of peak cholinesterase inhibition. RBC and brain cholinesterase activities were determined at 20, 40, 60, 120, 240, and 480 minutes after dosing in the adult (0.08 mg/kg) and PND 11 (0.01 mg/kg) groups (controls evaluated at 20 or 480 minutes). PND11 pups of the 0.02 mg/kg dose group were assessed at 40, 60, 120, and 240 minutes after dosing (controls evaluated at 60 minutes).

Results: All rats (both age groups) survived to scheduled sacrifice, and there were no clinical signs. Maximal RBC inhibition was observed at 20 minutes in adult females and at both 20 and 40 minutes in adult males. In the brain, maximal inhibition was observed at 40 minutes in both sexes of adult

rats. In the PND 11 pups, maximal RBC inhibition was observed 60 minutes (males) and 40 minutes (females) at 0.10 mg/kg and at 60 minutes (both sexes) at 0.02 mg/kg. Brain inhibition was maximal at 40-60 minutes in the male PND 11 pups and at 60 minutes in the female PND 11 pups. Based on the results of this time-course study, times of 40 minutes and 60 minutes were selected as the time of cholinesterase determination in the definitive dose-response study for adults and PND 11 pups, respectively.

Dose-Response Study (MRID47994305): In the definitive dose response study, aldicarb (99.8% a.i. ; Lot #: 1218200307) was administered once *via* gavage to 8 adult Crl: CD (SD) rats/sex/dose at dose levels of 0, 0.03, 0.05, 0.065, 0.08, 0.15, or 0.3 mg/kg, and to 8 PND 11 pups/sex/dose at dose levels of 0, 0.005, 0.01, 0.02, 0.04, or 0.08 mg/kg. Erythrocyte and brain cholinesterase activities were determined [(Hunter et al., 1997 modification of the Ellman reaction (Ellman, et al., 1961)] at the estimated time of peak-effect of 40 minutes post-dosing in adult rats and at 60 minutes post-dosing in PND 11 pups. Samples were maintained in an ice-water bath from point of collection until analysis for cholinesterase activity. Samples were analyzed within one hour of sample collection.

Results: *Clinical signs.* There were no treatment-related effects on mortality in either age group. All 0.3 mg/kg adult rats (both sexes) showed slight to moderate tremors of the limbs by 20 minutes post-dosing and at sacrifice at 40 minutes, and 3 adult males and 4 adult females in the 0.15 mg/kg group displayed slight tremors at 40 minutes. No tremors were observed in the PND 11 pups at any dose level, and none of the adult rats displayed tremors at 0.08 mg/kg, which was the only common dose level between the age groups and highest dose level in the PND 11 pups.

RBC Cholinesterase. PND 11 pups were more sensitive than the adult rats, based on a comparison of RBC cholinesterase inhibition. There was a dose-related decrease in RBC cholinesterase activity in the adult rats (both sexes), with the magnitude of the reduction (28%) at the lowest dose (0.03 mg/kg) in the females being biologically significant. An assessment of the adult sexes combined at 0.03 mg/kg showed 20% inhibition, which was statistically significant. At the two highest dose levels, nearly complete inhibition of RBC cholinesterase activity was observed in both sexes of adult rats (96%-99% at 0.15 mg/kg and 94%-97% at 0.3 mg/kg). The magnitude of the ChE activity at these two highest doses corresponds with the tremors that were observed in adults at either 20 or 40 minutes post-dosing. In the PND 11 pups, both sexes displayed a dose-related reduction in RBC cholinesterase activity, with the magnitude of the response being 28%-34% at 0.01 mg/kg and 85%-87% at the highest dose tested (0.08 mg/kg). At 0.005 mg/kg (lowest dose tested), ≈10% RBC cholinesterase inhibition was observed in the PND 11 pups.

Brain Cholinesterase. PND 11 pups were more sensitive than the adult rats, based on a comparison of brain cholinesterase inhibition. In the adult rat, a statistically significant reduction (dose-related) in brain cholinesterase activity was observed at dose levels of 0.05 mg/kg and above in both sexes. The magnitude of the decrease was 6%-9% at 0.05 mg/kg, 9%-10% at 0.065 mg/kg, 13%-18% at 0.08 mg/kg, 27%-28% at 0.15 mg/kg, and 43%-50% at 0.3 mg/kg. Brain cholinesterase activity was decreased at all doses except at the lowest dose (0.005 mg/kg) in the PND 11 pups (both sexes). The magnitude of the decrease was 8% at 0.01 mg/kg, 15%-16% at 0.02 mg/kg, 38%-44% at 0.04, and 59%-60% at 0.08 mg/kg in the PND11 pups (both sexes). Generally, pups were 4-8 fold more sensitive than the adult rats, depending on the sex and dose evaluated.

A benchmark dose analysis of the cholinesterase data (RBC and brain) was performed that provides both the BMD_{10} and $BMDL_{10}$ of adults and PND11 pups.

A separate benchmark dose analysis[1] of the definitive dose-response data demonstrated the following BMD_{10}s and $BMDL_{10}$s for adult cholinesterase:

	RBC BMD_{10}	RBC $BMDL_{10}$	Brain BMD_{10}	Brain $BMDL_{10}$
Adult females	0.0242 mg/kg	0.0144 mg/kg	0.0615 mg/kg	0.0498 mg/kg
Adult males	0.0228 mg/kg	0.0153 mg/kg.	0.0535 mg/kg	0.0484 mg/kg

A separate benchmark dose analysis[2] of the definitive dose-response data demonstrated the following BMD_{10}s and $BMDL_{10}$s for PND 11 pup cholinesterase:

	RBC BMD_{10}	RBC $BMDL_{10}$	Brain BMD_{10}	Brain $BMDL_{10}$
PND11 females	0.00731 mg/kg	0.00387 mg/kg	0.0136 mg/kg	0.0103 mg/kg
PND11 males	0.00477 mg/kg	0.00294 mg/kg	0.0143 mg/kg	0.0112 mg/kg

A ratio of the BMD_{10} adults to BMD_{10} PND11 pups provides a data derived FQPA factor.

RBC FQPA (female)
Adult BMD_{10}/PND11 BMD_{10} = 3.31

Brain FQPA (female)
Adult BMD_{10}/PND11 BMD_{10} = 4.52

RBC FQPA (male)
Adult BMD_{10}/PND11 BMD_{10} = 4.78

Brain FQPA (male)
Adult BMD_{10}/PND11 BMD_{10} = 3.74

These studies are classified as **acceptable/non-guideline**. These studies do not satisfy a guideline requirement for aldicarb. They satisfy the generic data call-in requirement for aldicarb for a comparative cholinesterase study in adult rats versus postnatal day (PND) 11 pups.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Aldicarb

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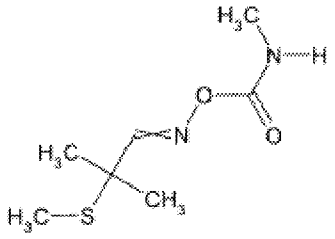
[1] *Refer to memo entitled "Statistical Review of Sielken, R. (2010) Benchmark Doses for Brain and RBC Acetylcholinesterase in Adult and Pups Exposed to Aldicarb" from B. Sarkar, dated July 1, 2010, D379831.*

[2] *Refer to memo entitled "Statistical Review of Sielken, R. (2010) Benchmark Doses for Brain and RBC Acetylcholinesterase in Adult and Pups Exposed to Aldicarb" from B. Sarkar, dated July 1, 2010, D379831.*

Description:	White crystalline powder
Lot #:	1218200307
Purity (w/w):	99.8% a.i.
Stability:	Expiration date: February 23, 2019; WIL Log # 090074
CAS #:	116-06-3
Structure:	Structure: 

2. **Vehicle** - deionized water

3. **Test animals**

Species:	Rat
Strain:	Crl:CD(SD)
Adult age/weight at study initiation:	adult rats 4-5 weeks at receipt; time-mated dams received on GD 10 or 11 (#21208: GD 11, 12, or 13); used to supply PND 11 pups; selected adults ≈7 weeks old; body weights: males: #21207 (231-281); 21208 (200-350); 21209 (198-262); #21210 (222-285) grams; females: #21207 (157-207); #21208 (150-250); #21209 (160-198) grams
Pup Age/weight at dosing:	11 days old; males: #21207 (20.9-28.6); 21208 (Phase II 23.5-31.7; Phase III 20.9-26.6); 21209 (22.1-27.7); #21210 (25.1-29.0) grams; females: #21207 (20.4-27.8); #21208 (Phase II 23.5-30.2; Phase III 21.0-26.6); #21209 (20.5-27.5) grams
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Each dam was housed with her litter in a nesting box during the post-natal period. Adults were individually housed in stainless steel, wire bottomed cages.
Diet:	Certified Rodent Diet #5002 (PMI Nutrition International, LLC.), *ad libitum*
Water:	Reverse osmosis treated (on-site) drinking water, *ad libitum*
Environmental conditions:	
Temperature:	22±3°C
Humidity:	50±20%
Air changes:	10/hr
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	Adults, ≥ 13- 23 days; pups 0-1 days

B. STUDY DESIGN

1. **Definitive study purpose** - This non-guideline study (MRID47994305; 21209) was undertaken to evaluate any differences between neonatal (postnatal day 11; PND 11) and adult rats with regard to cholinesterase activity as a biomarker for more general neurological effects. Preliminary studies (MRID47994302; 21207 and MRID47994304; 21208) were performed to determine the appropriate

dose levels for the time to peak effect study and for the definitive acute comparative dose response study (MRID47994305; 21209) with regard to inhibition of red blood cell (RBC) and brain cholinesterase. Another study (MRID47994303; 21210) assessed the effects of aldicarb on RBC and brain cholinesterase in male adult rats and male PND 11 pups at the same dose levels.

2. **In-life dates** - MRID 47994302: WIL 21207: Start: June 22, 2009; End: June 26, 2009
MRID 47994304: WIL 21208: Start: July 13, 2009; End: September 22, 2010
MRID 47994305: WIL 21209: Start: September 15, 2009; End: October 15, 2009
MRID 47994303: WIL 21210: Start: August 9, 2009; End: August 9, 2009

3. **Animal assignment and treatment –Dose-Response Study (MRID 47994305). Phase I (adult rats)**: There were 6 aldicarb-treated groups and one control group, each consisting of 8 adult rats/sex/group (Table 2). After randomization (based on body weight stratification randomized in a block design), these rats were randomized into 2 study replicates to allow for the reasonable conduct of cholinesterase assessments. Each dose group and sex was ≈equally represented within each study replicate. **Phase II (PND 11 pups):** Pups were randomly assigned to one of 5 groups, such that no more than 1 pup/sex/litter was assigned to each group (each group consisted of 8 pups/sex/dose). Pups were also randomized into 4 replicates to allow for reasonable conduct of all necessary study functions. Each dose group and sex was equally represented within each study replicate for dose administration. Adults and pups (on PND 11) received a single gavage dose at a volume of 5 mL/kg body weight.

Study design: Table 1 shows the objectives and treatment groups allocated for the four studies.

TABLE 1. Study Design for Cholinesterase Inhibition Studies on Aldicarb

MRID Study #	Dose(s) (mg/kg)	# rats/sex	Objective/Treatment/Termination
47994302 WIL 21207	Phase I (adult): 0.1, 0.3 Phase II (PND 11 pups): 0.025, 0.05, 0.1	6/sex 6/sex	Determine dose levels (dose-range finding) for the time of peak effect and dose-response studies for inhibition of RBC and brain cholinesterase. Single oral dose; clinical observations at 20 and 60 min post-dose; cholinesterase activity (brain and RBC) evaluated at 60 min post-dose (termination)
47994304 WIL 21208	Phase I (adult): 0.08 Phase II (PND 11 pups): 0.01 Phase III (PND 11 pups): 0.02	6/sex/time point 6/sex/time point 6/sex/time point	Determine time to peak inhibition (RBC and brain cholinesterase). Single oral dose on PND 11 (Phases II & III) or Day 0 adults (Phase I); six control rats/sex/phase terminated at 20 (Phases I & II), 60 (Phase III), or 480 (Phases I & II) minutes post- dose or six rats/sex/phase terminated at 20, 40, 60, 120, 240, or 360 min post-dose.
47994305 WIL 21209	Phase I (adult): 0.03, 0.05, 0.065, 0.08, 0.15, 0.3 Phase II (PND 11 pups): 0.005, 0.01, 0.02, 0.04, 0.08	8 rats/sex 8 rats/sex	Determine the dose-response for inhibition of RBC and brain cholinesterase in adult (Phase 1) and PND 11 (Phase II) rats. Single oral dose: Adult rats euthanized at 40 minutes post dose; PND 11 pups at 60 minutes post dose. Definitive Acute Comparative Cholinesterase Assay (CCA) study
47994303 WIL 21210	Phase I (adult): 0.01 and 0.04 Phase II (PND 11 pups): 0.01 and 0.04	6 males 6 males	Compare effects on RBC and brain cholinesterase (single dose) using same dose levels of aldicarb in adult and PND 11 male rats. Clinical observations prior to dose, ≈20 min post dose, prior to sacrifice; adult rats euthanized at 40 minutes post dose; PND 11 pups at 60 minutes post dose

PND = postnatal day

4. Dose selection and sampling time rationale; The dose levels and sampling times selected for the dose-response study (MRID 47994305) were based on the results of the three preliminary studies, described below (a, b, c). Aldicarb was administered once *via* gavage to 8 adult rats/sex/dose at dose levels of 0, 0.03, 0.05, 0.065, 0.08, 0.15, or 0.3 mg/kg, and to 8 PND 11 pups/sex/dose at dose levels of 0, 0.005, 0.01, 0.02, 0.04, or 0.08 mg/kg in the definitive dose-response study (MRID 47994305).

a) Dose selection rationale for time to peak effect study - In a dose range-finding study (MRID47994302; WIL 21207), 6 PND 11 pups/sex/dose were administered a single gavage dose of 0 (deionized water), 0.025, 0.05, or 0.1 mg/kg aldicarb and 6 adult (49-50 days old) rats/sex/dose were administered a single gavage dose of 0 (deionized water), 0.1 or 0.3 mg/kg aldicarb. All rats were euthanized ≈60 minutes post-dose. Slight whole body tremors and slight to moderate tremors of the limbs were observed in all adult rats at 0.3 mg/kg (≈20 minutes post dose) and at sacrifice (≈60 minutes post dose). Additionally, one 0.3 mg/kg adult male showed repetitive movement of the mouth just prior to sacrifice. Slight whole body tremors were observed in 2 male and 4 female PND 11 pups in the 0.1 mg/kg dose group and in one female PND 11 pup in the 0.05 mg/kg group at ≈20 minutes or just prior to sacrifice at ≈60 minutes post dose.

There was a dose-related, statistically significant, reduction in cholinesterase activity in both the brain and RBC compartments at all dose levels in both sexes and age groups (Tables 2 and 3). The PND 11 pups showed a slightly greater reduction in RBC cholinesterase activity at 0.1 mg/kg (males

↓93%/females ↓87%) than the adult rats (males ↓76%/females ↓64%) at the same dose level. Also at 0.1 mg/kg, PND 11 pups (both sexes) displayed a greater reduction in brain cholinesterase activity (↓67%) than the adult rats (≈↓10%). The magnitude of the reduction in brain cholinesterase activity was similar between the age groups at a dose level of 0.3 mg/kg in the adults (males ↓39 %/females ↓49%) compared to a dose level of 0.05 mg/kg (males ↓39 %/females ↓45%) in the PND 11 pups (6-fold difference in dose). The dose levels selected from this study for the time-to-peak effect study (MRID47994304; WIL 21208) were 0.08 mg/kg (adult rat) and 0.01 mg/kg (PND 11 pup). During protocol review, HED recommended a second dose be tested in the PND 11 group (phone conversation). Due to insufficient inhibition of cholinesterase activity at 0.01 mg/kg, an additional dose of 0.02 mg/kg was selected. Red blood cell and brain cholinesterase activities were assessed at 20, 40, 60, 120, 240, and 480 minutes after dose administration at these dose levels (MRID47994304; WIL 21208).

Table 2. Study Design and Results (**RBC**) of Range-Finding Study (MRID47994302; WIL 21207)

Dose (mg/kg)	**# of rats/sex**	**RBC Cholinesterase (U/L)**		**Sample Time (minutes post-dosing)**
		Males	**Females**	
Adults				
0	6	2348±450	2447±451	60
0.1	6	572±597**↓76%	873±711** ↓64%	60
0.3	6	30±43** ↓99%	27±41** ↓99%	60
PND 11 Pups				
0	6	4506±1498	3936±1135	60
0.025	6	1060±523** ↓77%	1323±613** ↓66%	60
0.05	6	812±864** ↓82%	1170±786** ↓70%	60
0.1	6	325±200** ↓93%	496±455** ↓87%	60

a Data obtained from Table 4 (pages 45-46) and Table 9 (pages 55-56) of the study report.
** $p \leq 0.01$; n = 6; mean ± s.d.

Table 3. Study Design and Results (**Brain**) of Range-Finding Study (MRID47994302; WIL 21207)

Dose (mg/kg)	# of rats/sex	Brain Cholinesterase (U/L)		Sample Time (minutes post-dosing)
		Males	Females	
Adults				
0	6	46132±2158	46162±2074	60
0.1	6	41808±1426** ↓9%	41307±3033** ↓11%	60
0.3	6	28338±2753** ↓39%	23772±2241** ↓49%	60
PND 11 Pups				
0	6	23963±1022	24765±596	60
0.025	6	18070±2289** ↓25%	18820±2110** ↓24%	60
0.05	6	14700±3699** ↓39%	13520±2715** ↓45%	60
0.1	6	7994±1013** ↓67%	8275±1366** ↓67%	60

a Data obtained from Table 4 (pages 45-46) and Table 9 (pages 55-56) of the study report.
** $p \leq 0.01$; n = 6; mean± s.d.

b) <u>Time point selection rationale</u> - In a time-course study (MRID47994304; WIL 21208), 6 rats/sex/group/time point were treated with single (gavage) doses of 0.08 mg/kg (adult rat), 0.01 mg/kg (PND 11 pups), or 0.02 mg/kg (PND 11 pups). RBC and brain cholinesterase activities were analyzed at sacrifice at 20, 40, 60, 120, 240, or 480 minutes post dose (Tables 4 and 5). All animals (both age groups) survived to scheduled sacrifice, and there were no clinical signs. In the **adult rats**, maximal RBC inhibition was present at 20 minutes (females) and a comparable response was observed in males at 20 minutes and 40 minutes post dose. **PND 11 pups** displayed a maximal RBC response at 60 minutes (males) and 40 minutes (females) at 0.01 mg/kg and a maximal response at 60 minutes (both sexes) following the 0.02 mg/kg dose. In the brain, maximal inhibition was present at 40 minutes in adult rats (both sexes) and at 60 minutes in PND 11 pups (both sexes). Regarding the time to peak effect, time points of 60 minutes and 40 minutes were selected for the PND 11 pups and adult rats, respectively, for the definitive dose-response study (MRID47994305; WIL 21209).

With the exception of the PND11 females, the RBC recovery half-lives were approximately 50-55 minutes. The RBC recovery half-life for female PND11 rats was estimated to be approximately 10 minutes, but this may be due to the unusual behavior of the AChE control values.

RBC Recovery Half-Lives (minutes)[3]		
Sex	Adult	PND11 Pups
Male	54.79	50.02
Female	54.45	9.82

With the exception of the PND11 females, the brain recovery half-lives were approximately 45-65 minutes. The brain recovery half-life for female PND11 rats was estimated to be approximately 128 minutes (roughly twice as long as the male PND11 pups), but this does not seem to be due to the unusual behavior of the AChE control values.

Brain Recovery Half-Lives (minutes)[3]		
Sex	Adult	PND11 Pups
Male	46.50	64.10
Female	57.54	127.80

3

3 Refer to memo entitled "Recovery Half-Lives for RBC and Brain Acetylcholinesterase in Adult and Pup Rats Exposed to Aldicarb" from B. Sarkar, dated July 12, 2010. D380046

Table 4. Time Course Study (MRID47994304; WIL 21208) - Mean (±SD) Cholinesterase Activity in **PND 11 Pups** Administered Aldicarb *via* Gavage (acute)[a]

Time post-dose (minutes)	Dose (mg/kg)	Red Blood Cells		Brain	
		cholinesterase (U/L)	% inhibition	cholinesterase (U/L)	% inhibition
Males					
20	0	3987±1278	-	24379±1017	-
	0.01	3728±774	6.5	24613±735	-
	0.02	-	-	-	-
40	0	-	-	-	-
	0.01	3826±677	4.0	23012±611*	5.6
	0.02	4050±1110	5.0 [C]	20694±969**	12.5
60	0 [C]	4262±1167		23638±974	-
	0.01	3294±1591	17.4	23073±1040	5.4
	0.02	2709±515*	36.4 [C]	19978±1790**	15.5
120	0	-		-	-
	0.01	3904±723	2.1	23168±628*	5.0
	0.02	3753±1111	11.9 [C]	20308±1593**	14.1
240	0	-	-	-	-
	0.01	4528±829	-	24190±1271	0.8
	0.02	6179±535**	-	23666±527	3.0
480	0	5193±1456	-	23756±417	-
	0.01	4587±863	11.7	24179±317	-
	0.02	-	-	-	-
Females					
20	0 [B]	3565±819	-	25444±1034	-
	0.01	3353±838	5.9	24422±619	4.0
	0.02	-	-	-	-
40	0	-	-	-	-
	0.01	2944±675	17.4	23745±1477*	6.7
	0.02	3109±478**	50.5 [C]	21522±1670*	8.7
60	0 [C]	6283±2095	-	23580±1212	-
	0.01	3529±364	1.0	23234±1162**	8.7
	0.02	2335±572**	62.8 [C]	20109±2083**	14.7
120	0	-	-	-	-
	0.01	4102±923	-	23393±1011**	8.1
	0.02	4704±701	25.1	21118±1768*	10.4
240	0	-	-	-	-
	0.01	3655±598	-	24706±1302	2.9
	0.02	6176±1666	1.7 [C]	24453±713	-
480	0 [B]	5240±1361	-	24604±633	-
	0.01	5417±1539	-	24129±557	1.9
	0.02	-	-	-	-

a Data were obtained from Table 19 (pages 89-94) and Table 27 (pages 109-112) of the study report. n = 6, except Phase I males at 40 minutes, Phase II males at 40 minutes, Phase II females at 40 minutes, Phase II females at 240 minutes when n=5 (sample clotted).

[B] Phase II control; [C] Phase III control (0.02 mg/kg); * p≤0.05; ** p≤0.01

Table 5. Time Course Study (MRID47994304; WIL21208). Mean (±SD) Cholinesterase Activity in **Adult Rats** Administered Aldicarb *via* Gavage (acute).

Time post-dose (minutes)	Dose (mg/kg)	Red Blood Cells		Brain	
		Cholinesterase (U/L)	% inhibition	Cholinesterase (U/L)	% inhibition
Males					
20	0	3658±529		50433±1938	
	0.08	958±635**	74	46454±1845**	8
40	0	-			
	0.08	951±328**	74	43246±1926**	14.
60	0	-			
	0.08	2084±481**	43	44942±2111**	11
120	0	-			
	0.08	2890±898	21	47913±2379	5
240	0	-			
	0.08	4333±647	-	49795±1824	1
480	0	4590±888		49520±2063	
	0.08	4694±466	-	48058±2064	3
Females					
20	0	4654±1017		48690±1340	
	0.08	1024±413**	78	44745±1221**	8
40	0	-			
	0.08	1299±425**	72	42913±1619**	12
60	0	-			
	0.08	1996±552**	57	44366±1684**	9
120	0	-			
	0.08	4291±872	8	46919±1723	4
240	0	-			
	0.08	4555±723	2	46832±5191	4
480	0	4795±842		48942±1263	
	0.08	4418±553	8	49835±827	-

a Data were obtained from Table 9 (pages 65-70) of the study report. n = 6; * p≤0.05; ** p≤0.01

c) <u>Comparative dose</u> - In a preliminary study (MRID47994303; WIL 21210) that compared cholinesterase activity between male adult rats and male PND 11 pups following acute oral exposure to aldicarb at the same dose levels (0.01 and 0.04 mg/kg), no brain or RBC cholinesterase inhibition was observed in the adult male rats, whereas in the PND 11 male pups, RBC cholinesterase was inhibited (↓32% and 74%) at both dose levels (dose-related) and brain cholinesterase was inhibited (↓37%) at the 0.04 mg/kg dose level (Table 6). Cholinesterase was measured at 60 minutes post dose in both age groups, and these data were considered during protocol review. The PND 11 male pup response in this preliminary study is comparable to that observed in the definitive dose-response study at these dose levels. These dose levels were not administered to the adult male rats in the definitive study; however, the response at 0.03 mg/kg (RBC: 12%; brain: 5%) and 0.05 mg/kg (RBC: 60%; brain: 9%) in the definitive study in the adult male rats is consistent for brain cholinesterase but not RBC cholinesterase at 0.04 mg/kg.

Table 6. Comparative Cholinesterase Values in Male Adult and PND 11 Pups.

	Control	0.01 mg/kg	0.04 mg/kg
PND 11 Pup RBC Cholinesterase Values (U/L)	5801±1493	3943±1257* (↓32%)	1484±647** (↓74%)
PND 11 Pup Brain Cholinesterase Values (U/L)	24193±1458	22814±1972 (↓6%)	15316±932** (↓37%)
Adult RBC Cholinesterase Values (U/L)	4238±483	4568±1052	4118±1117 (↓3%)
Adult Brain Cholinesterase Values (U/L)	48603±796	47988±2084	46805±2589 (↓4%)

Data from WIL 21210. Table 4 (page 41) & Table 9 (page 46) of the study report; mean ± s.d.; * $p<0.05$; ** $p<0.01$; n=6

5. Test substance preparation and analysis – Dose formulations were prepared by dilution of a stock solution, which was prepared once at concentrations of 0.3 mg/mL (Phase I) and 0.1 mg/mL (Phase II). A specified amount of vehicle (deionized water) was added to the appropriate amount of stock solution for each dose level. The dose formulations were prepared once as single formulations for each dose level, divided into aliquots for dispensation, and stored refrigerated. The dose formulations were stirred continuously throughout use. For Phase I, the pH of each dosing formulation was 6.68, 6.25, 6.39, 6.71, and 6.75 with increasing dose. For Phase II, the pH with increasing dose was 6.78, 6.24, 4.33, 4.28, and 4.44. Homogeneity, re-suspension homogeneity, and 10-day refrigerated storage stability of formulations in the range of 0.01 mg/mL to 0.06 mg/mL (MRID 47994302; WIL 21207) and homogeneity, re-suspension homogeneity, and 7-day refrigerated storage stability of formulations of 0.0002, 0.002, and 0.016 mg/mL (MRID 47994304; WIL 21208) were determined in the other studies and were not conducted in this definitive study.

Results:

Homogeneity analysis: The dosing formulations were inspected visually and were found to be visibly homogeneous and acceptable for administration. Homogeneity analyses in MRID 47994304 showed mean % of target to be 101%-114% for formulations of 0.0002, 0.002, and 0.016 mg/mL. For formulations of 0.005, 0.01, and 0.06 mg/mL (MRID 47994302), the mean % of target was 101%, 98-100%, and 98-102%, respectively.

Stability analysis: [HYPERLINK "\\\\\\insert"] Formulations of 0.0002, 0.002, and 0.016 mg/mL were 101%, 91-95%, and 91-97%, respectively, of the mean % of time zero after storage (refrigeration) for 3 to 7 days. After storage for 10 days, mean % of time zero after storage (refrigeration) was 102%.

Concentration analysis: The dosing formulations were within the testing laboratories standard operating procedures range for suspensions (85% to 115%) and were within the protocol requirement

for concentration acceptability (90% to 110% of target).

Concentration (% of target): Dose Response Study: Phase I: 96.9-106%; Phase II: 95.0-103%.

6. Statistics

Time to peak effect (MRID 47994304) and dose response (MRID 47994305) studies - All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test substance-treated group to the control group by sex. Each mean was presented with the standard deviation (S.D.), standard error (S.E.), and the number of animals (N) used to calculate the mean. Due to the use of significant figures and the different rounding conventions inherent in the types of software used, the means, standard deviations, and/or standard errors on the summary and individual tables may differ slightly. For each phase, mean body weights were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test substance-treated groups to the control group. All statistical analyses for red blood cell and whole brain cholinesterase data were conducted by BioSTAT Consultants, Inc., Portage, MI, using SAS version 9.1 software (SAS Institute, Inc., 2002-2003). Red blood cell and whole brain cholinesterase data were subjected to a parametric two-factor ANOVA for each study phase. The factors in the model were treatment group (TRT), SEX, and the TRT*SEX interaction. Dunnett's test was used to make pairwise comparisons of each individual treated group with the control group. If the TRT*SEX interaction was significant, Dunnett's test was conducted for each individual sex. If TRT*SEX was not significant, Dunnett's test was conducted overall across the pooled sexes only.

C. METHODS

1. Observations - Dose-Response Study (MRID 47994305)

a. Adults - All adult rats, including dams of offspring, were observed at least twice daily for mortality and moribundity. Clinical examinations were performed prior to dosing, ≈20 minutes following dose administration, and immediately prior to sacrifice (≈40 and 60 minutes post dose for Phases I and II, respectively).

b. Pups – One PND 0, pups were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. On PND 4, 10 pups per litter (5/sex when possible) were randomly selected to reduce variability among litters. Pups were fostered to litters, as necessary, to meet the appropriate sex ratio.

2. Body weight - Body weights of all adult rats and PND 11 pups were recorded initially and used as the basis of randomization into dose groups. Additionally, all rats were weighed on the day of dose administration to determine individual doses.

3. Cholinesterase activity determination - Blood samples and whole brains were collected from each rat at ≈ 40 minutes (Phase I) or 60 minutes (Phase II) following dose administration on study

day 0 (Phase I) or PND 11 (Phase II) for determination of cholinesterase activities as follows. Each rat was euthanized by carbon dioxide inhalation. If necessary, PND 11 pups were also anesthetized with isoflurane and euthanized by exsanguination during the blood collection procedure. Blood samples (at least 1 mL for adult animals and 0.5 mL for PND 11 pups) were collected from the inferior vena cava into chilled tubes containing sodium heparin as the anticoagulant and centrifuged for ≈ 10 minutes at ≈ 4°C. The plasma was discarded and the packed red blood cells (RBC) were diluted ≈ 1:20 (w:v) using 1% Triton X-100 solution (buffered). The contents of the tube were mixed (vortex mixer) and the RBC preparation was analyzed. Brains were excised from the skull and weighed. Whole brains were diluted ≈ 1:10 (w:v) using 1% Triton X-100 solution (unbuffered) and homogenized. The homogenate was centrifuged for ≈15 minutes at ≈ 4°C, and the supernatant was analyzed. RBC and whole brain cholinesterase activities were determined using an assay based on a modification (Hunter et al., 1997) of the Ellman reaction (Ellman et al., 1961), which uses acetylthiocholine as a substrate to measure total cholinesterase (i.e., the assay is not specific to acetylcholinesterase) *via* a photometric period (WIL SOP No. T5-146-2, dated 9/12/2006). All samples were maintained in an ice-water bath from the point of collection until analysis for cholinesterase activity. The time of each blood sample and brain collection was recorded and samples were analyzed within 1 hour of sample collection, based on the results of a previous validation study of carbamate inhibition of cholinesterase (Roegge, 2009, WIL-99420). Individual RBC cholinesterase values that were below the lower limit of quantification (20 U/L) following analysis were assigned a value of 10 U/L (mean of 0 U/L and 20 U/L) on the report tables.

II. RESULTS – Dose-Response Study (MRID 47994305)

A. OBSERVATIONS

1. Clinical signs of toxicity

a) Pups – No clinical signs were observed prior to dosing, at 20 minutes post dose, or immediately prior to sacrifice.

b) Adults - No clinical signs of toxicity were noted in the adults prior to dosing. At 0.3 mg/kg, all male and female rats showed slight to moderate tremors of the limbs at ≈20 minutes post dose and immediately prior to sacrifice. At 0.15 mg/kg, 3 males and 4 females displayed slight tremors of the limbs at ≈40 minutes post dose.

2. Mortality

a) Pups - All pups survived to scheduled termination.

b) Adults - All adult rats survived until scheduled termination.

B. BODY WEIGHTS - Pups and Adults - Body weights of the treated PND 11 pups and adult rats were comparable to their respective controls on the day of dose administration.

C. CHOLINESTERASE ACTIVITY – For the comparative dose-response cholinesterase inhibition study, the cholinesterase data for both compartments and age groups are summarized and presented below in Tables 7 and 8.

1. Erythrocyte (RBC) Cholinesterase - Pups. RBC cholinesterase activity was decreased (dose-related) in both sexes at 1 hour following acute exposure (males ↓ 11-87%/females ↓ 9-85%) to dose levels ranging from 0.005 to 0.08 mg/kg (Table 7). With the sexes combined, the reductions were 10-86% (not statistically significant at lowest dose). Three of the 8 lowest dose PND11 males (0.005 mg/kg) and 2 of the lowest dose PND11 females (0.005 mg/kg) displayed RBC cholinesterase values that were lower than the lowest control value (Summary Table 5). At dose levels of 0.01 mg/kg and above, all of the PND 11 males and 7-8 of 8 PND 11 females displayed RBC cholinesterase values that were lower than the lowest control value.

Adults. RBC cholinesterase activity was decreased (dose-related) in all dose groups in the adult rats of both sexes at 40 minutes following acute exposure (↓males 12-99%/↓females 28-96%) to dose levels ranging from 0.03 to 0.3 mg/kg (Table 8). With the sexes combined, the reductions were ↓20-98% (statistically significant at all dose levels). Females at the lowest dose tested (0.03 mg/kg) displayed a greater reduction (↓28.4%) than the males (↓11.8%), whereas at the next higher dose (0.05 mg/kg), males displayed the greater response (↓60% vs ↓39%). Six of the 8 lowest dose females (0.03 mg/kg) displayed RBC cholinesterase values that were lower than the lowest control value (Summary Table 6).

2. Brain Cholinesterase- Pups- Brain cholinesterase activity was decreased at all doses compared to the control values in the male (↓8-60%) and female (↓8-59%) PND 11 pups (Table 7). Although the magnitude of the mean decrease at the lowest dose level was less than 10%, an assessment of the individual brain cholinesterase data shows that 4 of the 8 male pups and 6 of the 8 female pups had values that were less than their respective control mean values (Summary Table 7). Furthermore, a BMD analysis will provide a level at which 10% inhibition was observed. Two of the lowest dose female PND 11 pups displayed 10-11% inhibition.

Adults – Brain cholinesterase activity was decreased at all doses except the lowest dose in the male (↓9-43%) and female (↓6-50%) adult rats (Table 8). At the lowest dose (0.03 mg/kg), the brain cholinesterase values in 5 of the 8 rats in each sex were lower than the lowest control value (Summary Table 8). At the common dose of 0.08 mg/kg, brain cholinesterase was reduced ↓13-18% in the adult rats compared to ↓59-60% in the PND 11 pups. Little or no reduction in brain cholinesterase activity (↓5%) was observed in the adult rats at 0.03 mg/kg compared to reductions of ↓16% (male pups)/↓15% (female pups) at 0.02 mg/kg and ↓44% (male pups) and ↓38% (female pups) at 0.04 mg/kg.

Table 7. PND 11 pup cholinesterase results

Sex	0 mg/kg	0.005 mg/kg	0.01 mg/kg	0.02 mg/kg	0.04 mg/kg	0.08 mg/kg
RBC Cholinesterase (U/L)						
PND 11 Males **%↓**	5104±795	4569±929 10.5	3654±1104 28.4	2153±518√ 57.8	931±170 81.8	654±254√ 87.2
range	4394-6246	3178-6100	2556-5992	1666-2836	546-1102 (5)	306-1096 (6)
PND 11 Females **%↓**	5279±974	4823±681√ 9	3493±771 34	2881±1017√ 45	1009±340 81	816±145 85
range	4352-7046	3966-6048	2524-4536	1352-4418	446-1690 (5)	606-1012 (7)
RBC Cholinesterase – Sexes Combined◄						
PND 11 M+F **%↓**	5191±864	4687±805 10	3573±924* 31	2517±863* 52	970±263* 81	741±212* 86
Brain Cholinesterase (U/L)						
PND 11 Males **%↓**	25613±846	25599±1026	23684±2084 8	21456±1426 16	14366±2275 44	10362±1177 60
range	24465-26729	23836-26680	19438-25619	18910-23295	9731-16920	8953-11865
PND 11 Females **%↓**	26081±1017	25633±1107	23940±830 8	22071±2190 15	16142±2206 38	10684±1745 59
range	24479-27605	23129-26698	22303-25026	18211-25002	11825-18729	8508-12934
Brain Cholinesterase – Sexes Combined◄						
PND 11 M+F **%↓**	25847±936	25616±1032	23812±1538*[2] 8	21763±1814* 16	15254±2351* 41	10523±1447* 59

Data obtained from Table 9 (pages 58-59) and Table 9A (pages 148-159) of the study report; * Dunnett's p-value # <0.001 or *[2]p<0.002; ◄Dunnett's test was used to make pairwise comparisons of each individual treated group with the control group. If the TRT*SEX interaction was significant, Dunnett's test was conducted for each individual sex. If TRT*SEX was not significant, Dunnett's test was conducted overall across the pooled sexes only; n = 8 rat/sex/group, except √, where n=7 (quantity not sufficient); (# <1000 U/L)

Table 8. Adult cholinesterase results

Sex	0 mg/kg	0.03 mg/kg	0.05 mg/kg	0.065 mg/kg	0.08 mg/kg	0.15 mg/kg	0.3 mg/kg
RBC Cholinesterase (U/L)							
Males **%↓**	2466±464	2175±789 12	993±423 60	786±432 68	303±222√ 88	24±31 99	83±90 97
Range	1852-3336	1188-3604	382-1752 (3)	42-1300 (4)	70-750 (7)	14-98 [5] (8)	56-198 [4] (8)
Females **%↓**	2411±315	1726±753 28	1473±568 39	777±532 68	610±420 75	97±116 96	153±116 94
Range	2054-2888	726-2760 (2)	414-2238 (1)	18-1418 (5)	230-1502 (7)	32-330 [3] (8)	104-302 [2] (8)
RBC Cholinesterase – Sexes Combined◄							
M+F **%↓**	2439±384	1950±780*[4] 20	1233±544* 49	781±468* 68	467±367* 81	60±90* 98	118±106* 95
Brain Cholinesterase (U/L)							
Males **%↓**	50448±1274	47858±1710 5	45805±1427* 9	46154±1829*[2] 9	41370±3154* 18	36816±2517* 27	28955±2276* 43
Range	48390-52765	45564-50828	44426-47769	43642-49465	36490-44623	33932-40447	26074-32964
Females **%↓**	50429±1460	47840±2819 5	47360±2454*[3] 6	45575±1642* 10	43792±3036* 13	36234±2600* 28	25295±3199* 508
Range	48581-52496	44157-52666	44525-51952	42525-47040	36716-46716	31791-39458	19792-28194
Brain Cholinesterase – Sexes Combined							
M+F **%↓**	50438±1324	47849±2252 5	46582±2099 8	45865±1705 9	42581±3242 16	36525±2490 28	27125±3281 46

Data obtained from Table 4 (pages 48-49) and Table A4 (pages 94-107) of the study report; n = 8 rat/sex/group, except √, where n=7 (one sample clotted); *statistically significant * Dunnett's p-value # <0.001 or *[2]p<0.002, or *[3] p<0.048, *[4]p<0.013; ◄Dunnett's test was used to make pairwise comparisons of each individual treated group with the control group. If the TRT*SEX interaction was significant, Dunnett's test was conducted for each individual sex. If TRT*SEX was not significant, Dunnett's test was conducted overall across the pooled sexes only; (# <1000 U/L); [# < LOQ]

D. BRAIN WEIGHT - Following acute oral exposure to aldicarb, brain weights were comparable among the groups in both age groups and sexes.

E. BENCHMARK DOSE (BMD) ANALYSIS: The registrant submitted a benchmark dose analysis of the cholinesterase findings (Sielken, R. (2010). Benchmark Doses for Brain and RBC Acetylcholinesterase in Adult and Pups Exposed to Aldicarb; MRID 47994306). The Chemistry and Exposure Branch (CEB) of HED has reviewed the submission to verify the benchmark dose (BMD) analysis of the cholinesterase data presented in MRID 47994306. CEB conducted a confirmatory analysis using EPA's Benchmark Dose Software (BMDS) 2.1.1, the same software used in MRID 47994306. CEB was able to reproduce the registrant's BMD analysis for the combined sexes. CEB also provided BMD estimates for each gender separately and calculated the associated FQPA factors (Table 3 from CEB memo, reproduced below).

Source	Sex	Target Organ	Adult		PND 11 Pups		FQPA Factor[A]
			BMD_{10}	$BMDL_{10}$	BMD_{10}	$BMDL_{10}$	
CEB	Male	Brain	0.0535	0.0484	0.0143	0.0112	3.741
		RBC	0.0228	0.0153	0.00477	0.00294	4.780
	Female	Brain	0.0615	0.0498	0.0136	0.0103	4.522
		RBC	0.0242	0.0144	0.00731	0.00387	3.310
Sielken & Associates[B]	Combined	Brain	0.0607	0.0517	0.0140	0.0116	4.336
		RBC	0.0223	0.0161	0.00573	0.00387	3.892

[A]Adult-to-Pup BMD_{10} ratio; [B]from MRID 47994306; from B. Sarkar memo, dated July 1, 2010, D379831.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS (from Report Summary) - Based on the time to peak effect studies in adult rats and PND 11 pups, the investigators concluded that 40 minutes post-dosing was the appropriate time to sample blood and brain tissue for cholinesterase activity in the adult phase and 60 minutes post-dosing was appropriate in the PND 11 pup phase of the comparative dose-response study. At the time of peak inhibition (40 minutes) in **adults**, reduced **RBC cholinesterase activity** was observed at 0.03, 0.05, 0.065, 0.8, 0.15, and 0.3 mg/kg. Reduced **whole brain cholinesterase activity** was observed at 0.05, 0.065, 0.08, 0.15, and 0.3 mg/kg in adult rats also. The effects on cholinesterase activity occurred in conjunction with tremors of the limbs at dose levels of 0.15 and 0.3 mg/kg. Based on these findings, the no-observed-effect level (NOEL) for RBC cholinesterase could not be established for adult male and female rats. The NOEL for whole brain cholinesterase activity for adult rats was determined to be 0.03 mg/kg. At the time of peak effect for juvenile (PND 11) rats, lower RBC and whole brain cholinesterase activities were noted for the 0.01, 0.02, 0.04, and 0.08 mg/kg groups. However, the effects on cholinesterase activity occurred without corresponding functional deficits. Based on these results, the **NOEL** was determined to be 0.005 mg/kg (both sexes) for RBC and whole brain cholinesterase activity for PND 11 pups.

B. REVIEWER COMMENTS - There were no deaths following acute oral exposure to aldicarb in either the adult rats or PND 11 pups. Clinical signs of toxicity consistent with cholinesterase inhibition (tremors) were observed only in the adult rats only at dose levels greater than those

administered to the PND 11 pups. At the highest dose tested in the PND 11 pups (0.08 mg/kg), the adult rats did not display tremors.

The results demonstrate that PND 11 pups are more sensitive than adult rats to the effects of aldicarb on inhibition of cholinesterase activity. Both sexes of PND 11 pups displayed significant brain cholinesterase inhibition (↓15%-60%) at dose levels of 0.02 mg/kg – 0.08 mg/kg, whereas a similar but lower level of brain inhibition (↓13%-50%) occurred in the adult rats at 0.08 mg/kg to 0.3 mg/kg. Significant RBC cholinesterase inhibition (>↓80%) was observed in the PND 11 pups at 0.04 mg/kg and above, whereas the adult rat displayed this level of RBC cholinesterase inhibition at 0.08 mg/kg and above. The time to peak effect of both RBC and brain cholinesterase was slightly longer for PND11 pups (40-60 minutes) compared to adults (20-40 minutes). With the exception of the PND11 females, the RBC recovery half-lives were approximately 50-55 minutes and the brain recovery half-lives were approximately 45-65 minutes. The brain recovery half-life for female PND11 rats was roughly twice as long (128 minutes) as the male PND11 pups. Although the RBC recovery half-life for female PND11 rats was estimated to be approximately 10 minutes, this may be due to the unusual behavior of the AChE control values.

The studies are classified as **acceptable/non-guideline.** These studies do not satisfy a guideline requirement for aldicarb. They satisfy the generic data call-in requirement for aldicarb for a comparative cholinesterase study in adult versus postnatal day (PND) 11 pups.

D. STUDY DEFICIENCIES – None that would impact study interpretation. The description of the cholinesterase assay procedures lacks sufficient details. The standard operating procedures (SOP Nos. T5-146-2, T5-166-4, and T5-070-9) did not provide a description of procedures to be used when replicate samples were not consistent and/or an expected response was not observed.

APPENDIX A - SUMMARY DISCUSSION

COMPARISON WITH PREVIOUS ACUTE CCA STUDY (adult rats): For comparison, the whole brain cholinesterase data from the 1999 Moser studies (MRID 45068601/MRID45145701) for the **adult rats** [Long-Evans Crl®LE) BR] following acute exposure are provided below in Appendix Table 1.

Appendix Table 1. MRID 45145701Cholinesterase Activity (Adult Long-Evans Rats)

Dose (mg/kg)	Males	Females
Whole Brain		
0	4.96±0.16	4.80±0.10
0.05	4.37±0.12 (↓12%)	4.30±0.12 (↓10%)
0.1	3.64±0.17 (↓27%)	3.78±0.18 (↓21%)
0.2	2.78±0.31 (↓44%)	2.49±0.27 (↓48%)
0.3	2.41±0.56 (↓51%)	2.19±0.19 (↓54%)
Whole Blood		
0	0.407±0.036	0.516±0.057
0.05	0.067±0.016 (↓84%)	0.079±0.014 (↓85%)
0.1	0.029±0.002 (↓93%)	0.044±0.008 (↓91%)
0.2	0.016±0.001 (↓96%)	0.022±0.077 (↓96%)
0.3	0.013±0.002 (↓97%)	0.023±0.005 (↓96%)

Data from MRID 45145701; mean± SEM; n=4; 1 hour post dose; nmoles substrate hydrolyzed/mg tissue/min

The brain cholinesterase inhibition in the adult rat from the Moser study is comparable to that observed in the current study at both common dose levels in both sexes (Appendix Table 2).

Appendix Table 2. Whole Brain Cholinesterase Activity – adult rats

Dose (mg/kg)	Long-Evans	Crl:CD
Males		
0.05	↓12%	↓9%
0.3	↓51%	↓43%
Females		
0.05	↓10%	↓6%
0.3	↓54%	↓50%

Also shown in Appendix Table 1 are the whole blood data from the Moser study. Although RBC cholinesterase activity data were not provided in the Moser study, the whole blood findings at 0.2 mg/kg (↓96%)and 0.3 mg/kg (↓96-97%) in the Moser study demonstrate nearly complete inhibition, which was also demonstrated in the RBC compartment in the current study at 0.15 (↓96-99%) and 0.3 mg/kg (↓94-97%). At 0.05 mg/kg, a greater response was observed in the Moser study in whole blood (↓84-85%) compared to (↓39-60%) in RBC compartment.

COMPARISON WITH PREVIOUS ACUTE CCA STUDY (PND 17 pups): A comparison of brain cholinesterase activity (% inhibition) in PND 11 (current study) and PND 17 pups (Moser study) following acute oral exposure is shown in Appendix Table 3. PND 11 pups displayed ≈40% inhibition (38-44%) at 0.04 mg/kg compared to a similar response (40-47%) in PND 17 pups at 0.1 mg/kg (2.5-fold difference).

Appendix Table 3. Brain Cholinesterase Inhibition (PND 11[A] and PND 17[B] pups)				
	Male		Female	
mg/kg	PND 11 pups	PND 17 pups	PND 11 pups	PND 17 pups
0	25613±846.3	3.95±0.34	26081±1017.0	3.80±0.19
0.005	25599±1026.3		25633±1107.4	
0.01	23684±2083.7 (↓8%)		23940±830.0 (↓8%)	
0.02	21456±1426.4 (↓16%)		22071±2190.2 (↓15%)	
0.04	14366±2274.5 (↓44%)		16142±2205.8 (↓38%)	
0.05		2.83±0.11 (↓28%)		2.89±0.23 (↓24%)
0.08	10362±1176.9 (↓60%)		10684±1744.5 (↓59%)	
0.1		2.38±0.27 (↓40%)		2.02±0.11 (↓47%)
0.2		1.04±0.17 (↓74%)		1.17±0.18 (↓69%)
0.3		0.64±0.07 (↓84%)		0.90±0.11 (↓76%)

[A]Crl:CD (SD) pups; units U/L; mean ±s.d.; n=6; 1 hour post dose
[B]Long-Evans pups; units nmoles substrate hydrolyzed/mg tissue/min; mean ±SEM; n=4; I hour post dose

Appendix Table 4. Acute Comparative Cholinesterase Inhibition (aldicarb) – MRID 47994305				
	Brain		Brain	
	Male adult	Male PND 11 pup	Female adult	Female PND 11 pup
Control	50448±1274.1	25613±846.3	50429±1460.2	26081±1017.0
Treated				
0.005		25599±1026.3		25633±1107.4
0.01		23684±2083.7 (↓8%)		23940±830.0 (↓8%)
0.02		21456±1426.4 (↓16%)		22071±2190.2 (↓15%)
0.03	47858±1709.6 (↓5%)		47840±2818.9 (↓5%)	
0.04		14366±2274.5 (↓44%)		16142±2205.8 (↓38%)
0.05	45805±1427.1 (↓9%)		47360±2454.3 (↓6%)	
0.065	46154±1828.9 (↓9%)		45575±1641.9 (↓10%)	
0.08	41370±3154.0 (↓18%)	10362±1176.9 (↓60%)	43792±3035.7 (↓13%)	10684±1744.5 (↓59%)
0.15	36816±2516.7 (↓27%)		36234±2600.2 (↓28%)	
0.30	28955±2276.2 (↓43%)		25295±3199.2 (↓50%)	
	RBC		RBC	
	Male adult	Male PND 11 pup	Female adult	Female PND 11 pup
Control	2466±464.0	5104±795.4	2411±315.4	5279±973.8
Treated				
0.005		4569±929.1 (↓11%)		4823±681.2 (↓9%)
0.01		3654±1104.2 (↓28%)		3493±771.1 (↓34%)
0.02		2153±517.5 (↓58%)		2881±1017.1 (↓45%)
0.03	2175±788.7 (↓12%)		1726±753.1 (↓28%)	
0.04		931±169.6 (↓82%)		1009±340.1 (↓81%)
0.05	993±422.9 (↓60%)		1473±568.2 (↓39%)	
0.065	786±431.8 (↓68%)		777±531.7 (↓68%)	
0.08	303±221.9 (↓88%)	654±253.8 (↓87%)	610±420.1 (↓75%)	816±145.1 (↓85%)
0.15	24±30.6 (↓99%)		97±116.2 (↓96%)	
0.30	83±89.5 (↓97%)		153±115.6 (↓94%)	

Data from Table 4 (pages 48-49) and Table 9 (pages 58-59) of the report; units U/L

Summary Tables 1- 4 and 9 provide comparisons of the cholinesterase data among the four studies and demonstrate a consistency in the findings.

APPENDIX A - SUMMARY TABLES

Summary Table 1. PND11 Rat Control Values Across Studies	
Males PND11 RBC	
21207[A] range-finding for time-course study	4506±1498
21208 (time-course) 20 minutes 60 minutes [A, B] 480 minutes	 3987±1278 4262±1167 5193±1456
21209♪ (dose-response)	5104±795
21210♪ (extra study with males only)	5801±1493
Females PND11 RBC	
21207 [A]	3936±1135
21208 20 minutes 60 minutes [A, B] 480 minutes	 3565±819 6283±2095 5240±1361
21209 [A]	5279±974
21210 [A]	-
Males PND11 Brain	
21207 [A]	23963±1022
21208 20 minutes 60 minutes [A, B] 480 minutes	 24379±1017 23638±974 23756±417
21209 [A]	25613±846
21210 [A]	24193±1458
Females PND11 Brain	
21207	24765±596
21208 20 minutes 60 minutes[A, B] 480 minutes	 25444±1034 23580±1212 24604±633
21209 [A]	26081±1017
21210 [A]	-

[A] 60 minutes post dose; [B]control run with Phase III (PND 11; 0.02 mg/kg)

Summary Table 2. Adult Rat Control Values Across Studies	
Males- Adult RBC	
21207 [A]	2348±450
21208 20 minutes 480 minutes	 3658±529 4590±888
21209 [A]	2466±464
21210 [A]	4238±483
Females- Adult RBC	
21207 [A]	2447±451
21208 20 minutes 480 minutes	 4654±1017 4795±842

Summary Table 2. Adult Rat Control Values Across Studies	
21209 [A]	2411±315
21210 [A]	NT
Males –Adult Brain	
21207 [A]	46132±2158
21208 20 minutes 480 minutes	 50433±1938 49520±2063
21209 [A]	50448±1274
21210 [A]	48603±796
Females –Adult Brain	
21207 [A]	46162±2074
21208 20 minutes 480 minutes	 48690±1340 48942±1263
21209 [A]	50429±1460
21210 [A]	NT

[A] **60 minutes post dose; NT females not tested**

Summary Table 3. PND 11 Pup Cholinesterase Values Across Studies at 3 Common Dose Levels				
PND 11 Pup RBC Cholinesterase Values				
Study	**Control**	**0.01 mg/kg**	**0.02 mg/kg**	**0.04 mg/kg**
Males				
21207	4506±1498	-	-	-
21208	3987±1278[A] 4262±1167[B]	3294±1591 (↓17%)	2709±515* (↓36%)	-
21209	5104±795	3654±1104 (↓28%)	2153±518 (↓58%)	931±169.6 (↓82%)
21210	5801±1493	3943±1257* (↓32%)	-	1484±647** (↓74%)
Females				
21207	3936±1135	-	-	-
21208	3565±819[A] 6283±2095[B]	2944±675 (17%)§	2335±572** (↓63%)	-
21209	5279±974	3493±771 (↓34%)	2881±1017 (↓45%)	1009±340.1 (↓81%)
21210	-	-	-	-
PND 11 Pup Brain Cholinesterase Values				
Males				
21207	23963±1022	-	-	-
21208	24379±1017[A] 23638±974[B]	23012±611* (6%)§	19978±1790** (↓16%)	-
21209	25613±846	23684±2084 (↓8%)	21456±1426 (↓16%)	14366±2274.5 (↓44%)
21210	24193±1458	22814±1972 (↓6%)	-	15316±932** (↓37%)
Females				
21207	24765±596	-	-	-
21208	25444±1034[A] 23580±1212[B]	23234±1162** (↓9%)	20109±2083** (↓15%)	-
21209	26081±1017	23940±830 (↓8%)	22071±2190 (↓15%)	16142±2205.8 (↓38%)
21210	-	-	-	-

(↓% inhibition at 60 minutes, except § @ 40 minutes post dose; [A]control for 0.01 mg/kg dose; [B] control for 0.02 mg/kg dose; * $p<0.05$; ** $p<0.01$; mean± s.d.;

Summary Table 4. Comparison of Brain Response at Common Dose Levels in PND 11 Pups (WIL 21207, WIL 21208, WIL 21209, WIL 21210).

Dose (mg/kg)	Study #	Brain Cholinesterase (U/L)		Sample Time (minutes post-dosing)
		Males	Females	
PND 11 Pups				
0	21208	24379±1017	25444±1034	60
0.01		23073±1040 (↓6%)	23234±1162** (↓9%)	60
0	21209	25613±846	26081±1017	60
0.01		23684±2084 (↓8%)	23940±830 (↓8%)	60
0	21210	24193±1458	-	60
0.01		22814±1972 (↓6%)	-	60
0	21208	23638±974	23580±1212	60
0.02		19978±1790** (↓15%)	20109±2083** (↓15%)	60
0	21209	25613±846	26081±1017	60
0.02		21456±1426[A] (↓16%)	22071±2190[A] (↓15%)	60
0	21207	23963±1022	24765±596	60
0.025		18070±2289** ↓25%	18820±2110** ↓24%	60
0	21209	25613±846	26081±1017	60
0.04		14366±2274.5[A] (↓44%)	16142±2205.8[A] (↓38%)	60
0	21210	24193±1458	-	60
0.04		15316±932** (↓37%)	-	60
0	21207	23963±1022	24765±596	60
0.05		14700±3699** ↓39%	13520±2715** ↓45%	60
0	21209	25613±846.3	26081±1017.0	60
0.08		10362±1176.9[A] (↓60%)	10684±1744.5[A] (↓59%)	60
0	21207	23963±1022	24765±596	60
0.1		7994±1013** ↓67%	8275±1366** ↓67%	60

** p≤0.01; mean± s.d.; n = 6; ↓% inhibition; [A]statistics not run on sexes separately

Summary Table 5. PND 11 Pup RBC Cholinesterase		
Dose	**Range (mean)**	**# < lowest control**
Males		
0 mg/kg	4394-6246 (5104)	
0.005 mg/kg	3178-6100 (4569)	3/8
0.01 mg/kg	2556-5992 (3654)	8/8
0.02 mg/kg	1666-2836 (2153)	8/8
0.04 mg/kg	546-1102 (931)	8/8
0.08 mg/kg	306-1096 (654)	8/8
Females		
0 mg/kg	4352-7046 (5279)	
0.005 mg/kg	3966-6048 (4823)	2/8
0.01 mg/kg	2524-4536 (3493)	7/8

Summary Table 5. PND 11 Pup RBC Cholinesterase		
Dose	**Range (mean)**	**# < lowest control**
0.02 mg/kg	1352-4418 (2881)	7/8
0.04 mg/kg	446-1690 (1009)	8/8
0.08 mg/kg	606-1012 (816)	8/8

Summary Table 6. Adult Female RBC Cholinesterase		
Dose	**Range (mean)**	**# < lowest control**
0 mg/kg	2054-2888 (2411)	-
0.03 mg/kg	726-2760 (1726)	6/8
0.05 mg/kg	414-2238 (1473)	7/8
0.065 mg/kg	18-1418 (777)	8/8
0.08 mg/kg	230-1502 (610)	8/8
0.15 mg/kg	32-330 (97)	8/8
0.3 mg/kg	10-302 (153)	8/8

Summary Table 7. PND 11 Pup Brain Cholinesterase		
Dose	**Range (mean)**	**# < control mean**
Males		
0 mg/kg	24465-26729 (25613)	2/8
0.005 mg/kg	23836-26680 (25599)	4/8
0.01 mg/kg	19438-25619 (23684)	7/8
0.02 mg/kg	18910-23295 (21456)	8/8
0.04 mg/kg	9731-16920 (14366)	8/8
0.08 mg/kg	8953-11865 (10362)	8/8
Females		
0 mg/kg	24479-27605 (26081)	3/8
0.005 mg/kg	23129-26698 (25633)	6/8
0.01 mg/kg	22303-25026 (23940)	8/8
0.02 mg/kg	18211-25002 (22071)	8/8
0.04 mg/kg	11825-18729 (16142)	8/8
0.08 mg/kg	8508-12934 (10684)	8/8

Summary Table 8. Adult Brain Cholinesterase		
Dose	**Range (mean)**	**# < control mean**
Males		
0 mg/kg	48390-52765 (50448)	-
0.03 mg/kg	45564-50828 (47858)	5/8
0.05 mg/kg	44426-47769 (45805)	8/8
0.065 mg/kg	43642-49465 (46154)	7/8
0.08 mg/kg	36490-44623 (41370)	8/8
0.15 mg/kg	33932-40447 (36816)	8/8
0.3 mg/kg	26074-32964 (28955)	8/8
Females		
0 mg/kg	48581-52496 (50429)	-
0.03 mg/kg	44157-52666 (47840)	5/8
0.05 mg/kg	44525-51952 (47360)	6/8
0.065 mg/kg	42525-47040 (45575)	8/8
0.08 mg/kg	36716-46716 (43792)	8/8
0.15 mg/kg	31791-39458 (36234)	8/8
0.3 mg/kg	19792-28194 (25295)	8/8

Summary Table 9. Adult Brain Cholinesterase Results Across Studies										
Study	**0**	**0.01**	**0.03**	**0.04**	**0.05**	**0.065**	**0.08**	**0.1**	**0.15**	**0.3**
Males										
21207[A]	46132±2158							41808±1426**↓10		28338±2753**↓39
21208										
20	50433±1938						46454±1845**↓8%			
40	-						43246±1926**↓14%			
60	-						44942±2111**↓11%			
120	-						47913±2379			
240	-						49795±1824			
480	49520±2063						48058±2064			
21209 [B]	50448±1274		47858±1710		45805±1427↓9	46154±1829↓9%	41370±3154 ↓18%		36816±2517↓27	28955±2276↓43
21210 [B]	48603±796	47988±2084		46805±2589						
Females										
21207[A]	46162±2074							41307±3033**↓11		23772±2241**↓49
21208										
20	48690±1340						44745±1221**↓8%			
40	-						42913±1619**↓12%			
60	-						44366±1684**↓9%			
120	-						46919±1723			
240	-						46832±5191			
480	48942±1263						49835±827			
21209 [B]	50429±1460		47840±2819		47360±2454 ↓6%	45575±1642↓10	43792±3036↓13		36234±2600 ↓29	25295±3199↓52
21210 [B]	-	-		-						

mean±s.d.; [A] 60 minutes post dose; [B]40 minutes post dose; [C] minutes post dose; * and ** statistically significant; ↓% magnitude of inhibition

21209 (Phase 1 adult RBC cholinesterase, Table 4, pages 48-49); 21207 (Phase 1 Adult RBC/brain Table 4, pages 45-46);

Summary Table 10. Adult RBC Cholinesterase Results Across Studies										
Study	**0**	**0.01**	**0.03**	**0.04**	**0.05**	**0.065**	**0.08**	**0.1**	**0.15**	**0.3**
Males										
21207[A]	2348±450							572±597**↓76		30±43**↓99
21208										
20 [C]	3658±529						958±635**↓74			
40	-						951±328**↓74			
60	-						2084±481**↓43			
120	-						2890±898↓21			
240	-						4333±647			
480	4590±888						4694±466			
21209 [B]	2466±464		2175±789↓12		993±423↓60	786±432↓68	303±222 ↓88		24±31↓99	83±90 ↓97
21210 [B]	4238±483	4568±1052		4118±1117						

Females										
21207[A]	2447±451							873±711**↓64		27±41**↓99
21208										
20[C]	4654±1017						1024±413**↓78			
40	-						1299±425**↓72			
60	-						1996±552**↓57			
120	-						4291±872			
240	-						4555±723			
480	4795±842						4418±553			
21209[B]	2411±315		1726±753↓28		1473±568↓39	777±532↓68	610±420↓75		97±116↓96	153±116↓94
21210[B]	-	-		-						

[A] **60 minutes post dose;** [B] **40 minutes post dose;** [C] **minutes post dose**

Summary Table 11. PND 11 Pup RBC Cholinesterase Results Across Studies									
Study	**0**	**0.005**	**0.01**	**0.02**	**0.025**	**0.04**	**0.05**	**0.08**	**0.1**
Males									
21207[A]	4506±1498				1060±523**↓77		812±864**↓82		325±200**↓93
21208									
20[B]	3987±1278		3728±774 *↓7	-					
40	-		3826±677	4050±1110					
60√	4262±1167		3294±1591 ↓17	2709±515*↓36					
120	-		3904±723	3753±1111↓12					
240	-		4528±829	6179±535**					
480	5193±1456		4587±863 ↓12	-					
21209[A]	5104±795	4569±929	3654±1104↓28	2153±518 ↓58		931±170↓82		654±254↓87	
21210[A]	5801±1493		3943±1257*			1484±647**			
Females									
21207[A]	3936±1135				1323±613**↓66		1170±786**↓70		496±455**↓87
21208									
20[B]	3565±819		3353±838 ↓6	-					
40	-		2944±675 ↓17	3109±478**↓13					
60√	6283±2095		3529±364	2335±572**↓63					
120	-		4102±923	4704±701 ↓25					
240	-		3655±598	6176±1666					
480	5240±1361		5417±1539	-					
21209[A]	5279±974	4823±681 ↓9	3493±771↓34	2881±1017↓46		1009±340↓81		816±145↓85	
21210[A]	-		-			-			

[A] **60 minutes post dose**; [B] **minutes post dose;** * p<0.05; ** p<0.01; mean± s.d.; √control run with Phase III (0.02 mg/kg) PND 11 pups; ↓% inhibition

Summary Table 12. PND 11 Pup Brain Cholinesterase Results Across Studies									
Study	**0**	**0.005**	**0.01**	**0.02**	**0.025**	**0.04**	**0.05**	**0.08**	**0.1**
Males									
21207 [A]	23963±1022				18070±2289**↓25		14700±3699**↓39		7994±1013**↓67
21208									
20 [B]	24379±1017		24613±735	-					
40	-		23012±611*↓6	20694±969**↓13					
60	23638±974		23073±1040 ↓5	19978±1790**↓16					
120	-		23168±628 *↓5	20308±1593**↓14					
240	-		24190±1271	-					
480	23756±417		24179±317	23666±527-					
21209 [A]	25613±846	25599±1026	23684±2084↓8	21456±1426↓16		14366±2275↓44		10362±1177↓60	
21210 [A]	24193±1458		22814±1972			15316±932**			
Females									
21207 [A]	24765±596				18820±2110**↓24		13520±2715**↓45		8275±1366**↓67
21208									
20 [B]	25444±1034		24422±619	-					
40	-		23745±1477*↓7	21522±1670*↓9					
60	23580±1212		23234±1162**↓9	20109±2083**↓15					
120	-		23393±1011**↓8	21118±1768*↓10					
240	-		24706±1302	24452±71					
480	24604±633		24129±557	-					
21209 [A]	26081±1017	25633±1107	23940±830	22071±2190↓15		16142±2206↓38		10684±1745↓59	
21210 [A]	-		-			-			

[A] **60 minutes post dose;** [B] **minutes post dose;** * p<0.05; ** p<0.01; mean± s.d.; ↓% inhibition